Electroencephalography and clinical Neurophysiology, 1988, 69: 171–178
Elsevier Scientific Publishers Ireland, Ltd.

EEG 03359

The effects of nitrous oxide on P300 and reaction time

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(Accepted for publication: 24 June 1987)

Summary This experiment investigated the effects of 3 concentrations (15%, 25% and 35%) of nitrous oxide (N_2O) on reaction time (RT) and P300 latency and amplitude. Ten subjects responded to visually presented male or female names in an oddball paradigm with accuracy controlled at a high level. The results were analysed on a single-trial basis. Nitrous oxide increased both RT and P300 latency in a dose-related manner and these variables showed a strong between-dose correlation ($r = 0.67$). Nitrous oxide also decreased P300 amplitude but only up to the 25% dose. The between-dose correlation for P300 amplitude and RT was negligible ($r = -0.14$). These results suggest that P300 latency is an index of N_2O narcosis and are interpreted as indicating that narcosis involves the slowing of stimulus evaluation processes.

Key words: P300; Nitrous oxide; Reaction time; Narcosis

The effects of sub-anaesthetic doses of nitrous oxide (N_2O) include subjective symptoms, such as euphoria, as well as increased reaction time (RT), decreased accuracy and amnesia (Fowler et al. 1985). Evidence regarding the effects of N_2O on exogenous components of the event-related potential (ERP) is not clear cut (see Frost 1985, for a review). Amplitudes of auditory ERPs are decreased but visual ERPs appear more resistant, although Fenwick et al. (1984) recently found decreases in some components which were unaccompanied by latency shifts. With regard to painful stimuli, Benedetti et al. (1982) have reported an inverse N_2O dose-response relationship between the amplitude of some early ERP components and subjective reports of pain. On the other hand, attempts to relate N_2O induced decreases in the amplitude of early ERP components to an objective performance measure, RT, have not met with great success (Jarvis and Lader 1971; Fenwick et al. 1979). Nitrous oxide also appears to decrease the amplitude of P300 (Fenwick et al. 1979), a late component of the ERP which has been related to several aspects of cognitive functioning.

P300 amplitude is influenced by a variety of experimental variables (Rösler 1983). Recently, it has been proposed that these variables can be categorized along 3 dimensions: (1) subjective probability, (2) stimulus meaning, and (3) information transmission (Johnson 1986). In this view, P300 amplitude varies inversely with subjective probability and directly with stimulus meaning and information transmission. P300 latency is also sensitive to processing demands and it has been demonstrated that P300 latency and RT are related under circumstances which suggest that P300 indexes stimulus-evaluation time rather than the time to select and execute a response (Kutas et al. 1977; Duncan-Johnson and Kopell 1981; McCarthy and Donchin 1981, 1983; Magliero et al. 1984). If P300 latency times some mental events, then it might be useful as an index of narcosis induced by N_2O since N_2O is reported to slow information processing (Fowler et al. 1985).

The purpose of the present experiment was to determine the effects of N_2O on P300 latency and amplitude and the relationship between these ef-

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fects and a behavioural measure of narcosis, RT. The oddball paradigm with the fixed-name procedure of Kutas et al. (1977) was employed. The stimuli were highly discriminable and accuracy of responding was controlled at a high level in order to maximize the coupling between P300 latency and RT and to prevent any confounding due to variations in accuracy across conditions (Pfefferbaum et al. 1983). The data were analysed on a single trial basis to avoid artifacts associated with averaging (Ruchkin and Glaser 1978).

Methods

Subjects

Ten normal or corrected-vision right-handed volunteers between the ages of 21 and 28 were paid for their participation in the experiment. They were medically examined and signed an informed consent document.

Administration of breathing mixtures

The experimental breathing mixtures consisted of 3 concentrations of N_2O (15%, 25% and 35%), balanced with oxygen and stored in high pressure cylinders. Air stored in the same manner was used as a control. The bottles were connected to a manifold and switching arrangement which allowed the selection of any mixture. After pressure reduction, the mixture was metered to a Douglas bag connected via large diameter (3.4 cm internal diameter) hosing to a non-rebreathing oro-nasal aviation oxygen mask (Canadian Forces Model A13A) worn by the subject. This arrangement minimized breathing resistance and the creation of possible movement artifacts in the EEG.

Stimulus presentation

The stimuli were pairs of 5-letter male and female given names. The names in each pair were presented singly a total of 150 times in random order so that the probability of occurrence was 80% for the male name (high probability) and 20% for the female name (low probability). Seven different pairs were used, two for practice and five for the experiment proper. Each name was displayed for 1200 msec in upper-case letters on a computer screen with an inter-stimulus interval of 700 msec. The visual angle subtended was 2.2°. Intensity was fixed at a value judged to be comfortable and easily discriminable by pilot subjects. The screen was viewed from a comfortable reclining chair and the task was to indicate whether a male or female name was displayed by pushing either a left or right button (Microswitch Model PK 85022) mounted on each armrest. RT was defined as the latency between onset of a stimulus and button depression.

Recording

EEG activity was recorded inside a Faraday cage at Cz and Pz electrode sites of the 10-20 system. The electrooculogram (EOG) was recorded horizontally and vertically by electrodes affixed to the outer canthi of both eyes and supra- and sub-orbitally to the dominant eye, respectively. All electrodes were Ag-AgCl with an impedance of 5 kΩ or less, referenced to linked earlobes. The subjects were grounded with an electrode on the forehead. Gould pre-amplifiers (Model 11-5407-58) and amplifiers (Model 13-4615-58) were used with a bandpass of 0.05–30 Hz (−3 dB, 6 dB/octave rolloff). The EEG was digitized every 5 msec for a 1500 msec epoch with a 100 msec prestimulus baseline and stored by a PDP 11/04 computer which also controlled stimulus presentation and collected the RTs. A pen recorder (Gould Model 2400S) was used to monitor both the EEG and the EOG. Trials in which the EOG exceeded 45 μV in amplitude were discarded. The system was calibrated with a 2 Hz sine wave over the range 0–50 μV.

Quantification of P300

The single trial wave forms from Cz and Pz were filtered using a fourth-order zero-phase digital filter with an upper cut-off frequency of 4 Hz. The latency and amplitude of P300 were determined using the Woody filter technique with a search window extending from 250 msec to 650 msec (Woody 1967). To validate this technique, the filtered wave forms from Cz were hand scored using the criteria specified for P300 by Warren and Wideman (1983). The results from the two methods were virtually identical and only low-

probability data derived from the Woody technique are reported in this paper. With either method of measurement, high-probability P300 could not be identified consistently or reliably for scoring on a single trial basis.

Design and procedure

A repeated measures design was used in which air was used as a control both before and after the 3 doses of N_2O for a total of 5 conditions. For each subject, a different pair of names was used in each condition. The order of presentation of the 5 pairs of names was randomized and the order of administration of the 3 doses of N_2O was partially counterbalanced by presenting 6 subjects with the doses in a fully counterbalanced order and the remaining 4 subjects with 4 of the possible 6 dose orders. The assignment of high- and low-probability names to left or right responses was fully counterbalanced between subjects.

Prior to the experiment, the subjects were trained on the task while breathing both air and 35% N_2O. They were judged to be trained when: (1) obvious movement artifacts disappeared from the EEG; (2) eye blinks and eye movements were minimized during the collection of EEG data; and (3) responding was stable and as fast as possible while maintaining an error rate of between 0.67% and 2% (an error constituted pressing the wrong button).

In the experiment, the subjects were tested individually in a single session which lasted approximately 80 min. The oro-nasal mask was donned and eye movements were calibrated with respect to the centre of the stimulus display. Then the 5 conditions were performed in succession with a 15 min waiting period between each one to stabilize brain tissue saturation (Fowler et al. 1983). If an error rate of 2% was exceeded, the data were discarded and the run repeated. This occurred on two occasions. In addition, the first 10 trials in each condition were regarded as a warm-up and discarded.

Results

The error rate was very low and was comparable across conditions (Table I). This low rate prevented any meaningful assessment of error RTs which are therefore not considered further. The percentage of trials eliminated due to eye movements was also comparable across conditions (Table I). Furthermore, the eye movement records for trials that were not eliminated from the analysis revealed no apparent differences between air and N_2O.

TABLE I

Mean error rate for reaction time (RT) and mean percentage of trials eliminated due to eye movements. Based on 140 trials.

Breathing mixture	Air 1	Nitrous oxide (%)			Air 2
		15	25	35	
RT error rate (%)	1.2	0.6	0.7	1.2	0.5
Trials eliminated (%)	16	14	14	15	13

An averaged EEG wave form was calculated for each subject. The superimposed wave forms are illustrated in Fig. 1 for the first air control and the 35% N_2O condition. With either breathing mixture, the low-probability P300 is clearly identifiable but the high-probability P300 is much smaller or virtually absent in most cases.

The frequency histograms of low probability scores for RT, and for the latency and amplitude of single trial P300, were inspected for each subject in each condition. The RT and P300 latency distributions appeared to parallel one another closely in terms of both shape and variability. On the other hand, the P300 amplitude distributions were less consistent in shape and notably more variable. This latter observation was documented by calculating the coefficient of variation (CV) for the first air condition and 35% N_2O. For air, mean CV was 9% for RT, 10% for P300 latency (Cz), and 42% for P300 amplitude (Cz). For N_2O the corresponding values were 12%, 11% and 44%, respectively.

The effects of N_2O on RT and on single trial P300 latency and amplitude are summarized in Fig. 2. A 2-way repeated measures analysis of variance on the RT data in Fig. 2A revealed that probability level (F (1, 9) = 59.04, $P < 0.001$) and breathing mixture (F (4, 36) = 18.72, $P < 0.0001$) were significant but not the interaction. These results indicate that RT was faster to the

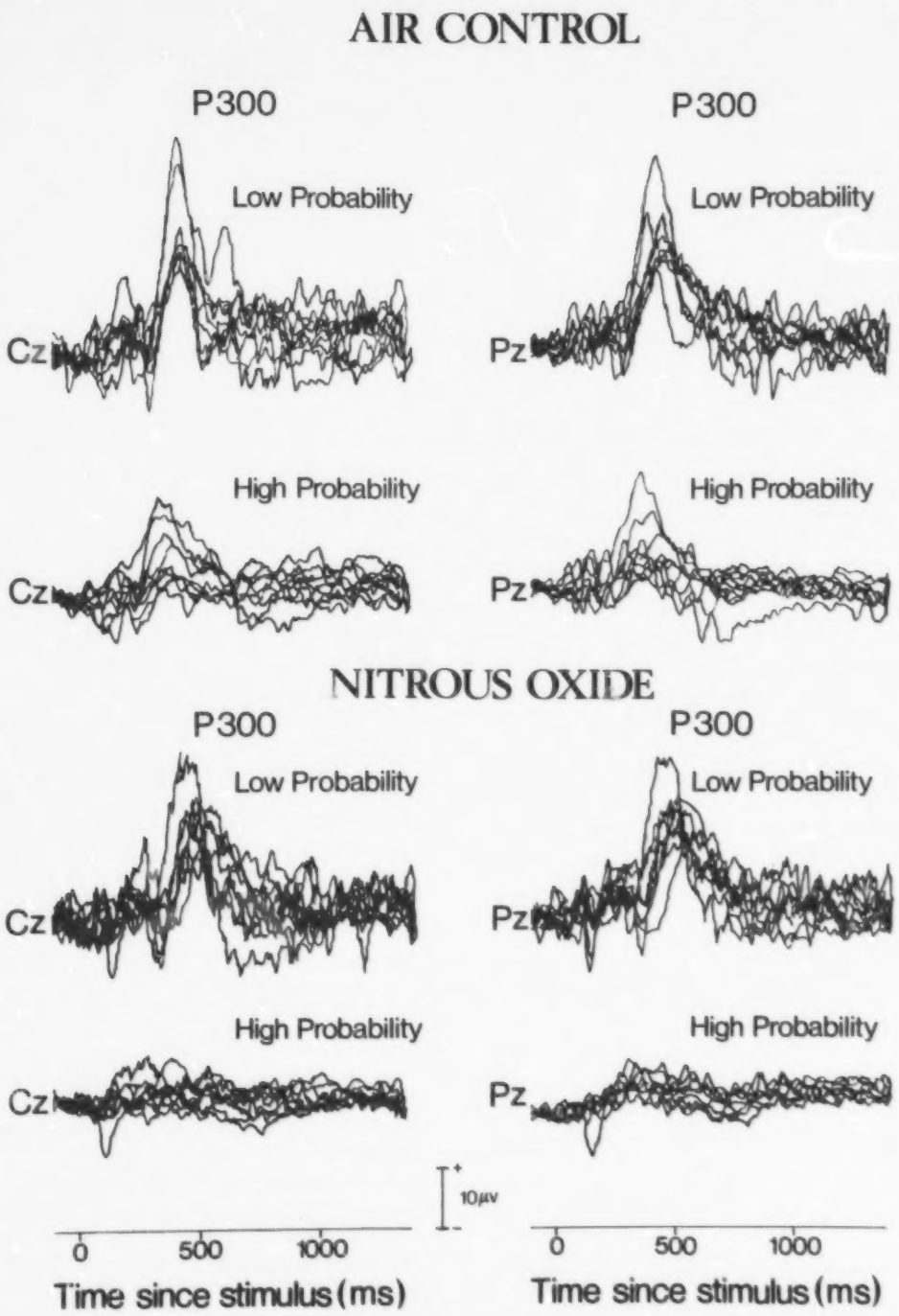

Fig. 1. Averaged event-related potentials for the first air control and 35% nitrous oxide as a function of stimulus probability and electrode site. The wave forms from each of the 10 subjects are superimposed to show variance across individuals.

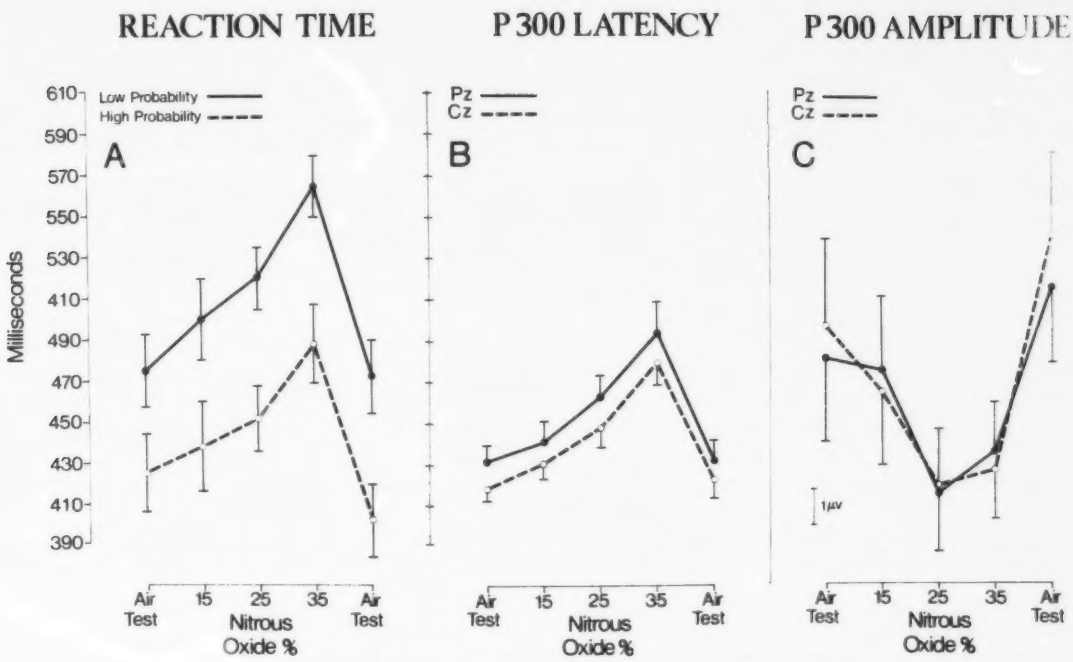

Fig. 2. A: mean reaction time as a function of nitrous oxide (N_2O) dose and stimulus probability. B: mean P300 latency to the low-probability stimulus as a function of N_2O dose and electrode site. C: mean P300 amplitude to the low-probability stimulus as a function of N_2O dose and electrode site. Bars represent S.E.M.

TABLE II

Within-dose and between-dose correlations (r) for reaction time (RT), P300 latency and P300 amplitude. Values are means based on the individual single trial correlations for each subject (N = 10).

Electrode	Breathing mixture				
	Air 1	Nitrous oxide (%)			Air 2
		15	25	35	
Within doses					
RT and P300 amplitude					
Cz	−0.02	−0.07	0.01	−0.06	0.01
Pz	−0.02	−0.04	0.00	−0.06	−0.05
P300 amplitude and P300 latency					
Cz	−0.06	−0.05	0.04	−0.01	0.09
Pz	−0.07	−0.01	0.04	0.14	−0.05
RT and P300 latency					
Cz	0.61	0.60	0.49	0.56	0.73
Pz	0.52	0.57	0.57	0.51	0.67

Between doses	Electrode	
	Cz	Pz
RT and P300 amplitude	−0.14	−0.15
P300 amplitude and P300 latency	−0.08	−0.05
RT and P300 latency	0.67	0.63

high-probability stimulus (441 vs. 506 msec). RT did not differ significantly between the first and second air controls (Duncan's test). As expected, RT slowed as the dose of N_2O increased. This slowing could be detected reliably at the intermediate dose (air 1 vs. 25%, $P < 0.01$) and was also detected at the lowest dose when the low-probability condition was considered alone (air 1 vs. 15%, $P < 0.01$). Fig. 2B shows that N_2O slowed P300 latency in a similar manner to RT although P300 preceded RT to the low-probability stimulus by approximately 70 msec. An analysis of variance on these data revealed that electrode site (F (1, 9) = 21.57, $P = 0.001$) and breathing mixture (F (4, 36) = 30.42, $P < 0.0001$) were significant but not the interaction. Latency was faster at Cz than Pz (440 vs. 452 msec). The 2 air controls were not different from one another and slowing could be detected at the lowest dose of N_2O (air 1 vs. 15%, $P < 0.01$).

Fig. 2C indicates that N_2O decreased P300 amplitude but the dose-response function for this variable differs from the one found for RT and P300 latency. In particular, P300 amplitude reached a minimum at 25% rather than 35%, the size of this change averaging 4 μV. An analysis of variance on these data revealed that breathing mixture (F (4, 36) = 7.03, $P = 0.0003$) and the breathing mixture × electrode site interaction (F (4, 36) = 2.76, $P = 0.04$) were significant. Amplitude at Pz and Cz in the first air control, and at Pz in the second control, did not differ. In the latter condition, amplitude at Pz was less than at Cz ($P < 0.05$), thus accounting for the interaction. The decrease in amplitude produced by N_2O could be detected reliably at the 15% dose for Cz ($P < 0.01$) but not Pz ($P < 0.10$) although the latter was clearly different at 25% ($P < 0.01$). As the dose of N_2O was increased from 25% to 35%, there was no significant change in amplitude at either Cz or Pz.

Within-dose and between-dose Pearson product-moment correlations for RT, P300 latency and P300 amplitude are presented in Table II. These values are means based on the individual single trial correlation for each subject. The pattern of within-dose correlations indicates that virtually no relationship exists between either RT and P300 amplitude or P300 amplitude and P300 latency. On the other hand, RT and P300 latency are substantially related with correlations ranging from 0.49 to 0.73. The between-dose correlations show a pattern similar to that described above for the within-dose correlations. While RT and P300 amplitude and P300 amplitude and P300 latency show little or no relationship, there is a substantial correlation between RT and P300 latency.

Discussion

In this experiment, the within-dose correlations between RT and P300 latency were substantial while those for RT and P300 amplitude were negligible. Furthermore, P300 latency preceded RT by about 70 msec. These results are consistent with previous findings when high accuracy is emphasized and the task involves a familiar name discrimination with a 20/80 probability structure (Kutas et al. 1977; McCarthy and Donchin 1983; Pfefferbaum et al. 1983). The present results are also consistent with other data showing that RT is slowed by N_2O in a dose-dependent manner (Fowler et al. 1985).

The major finding of this experiment was that P300 latency was slowed by N_2O in a dose-dependent manner at both Cz and Pz. This slowing was closely related to RT, as indicated by the substantial single trial correlation and the similarity in shape of both the frequency distributions and dose-response functions. However, these two variables differed in one respect — RT was slowed more than P300. At the 35% dose, this slowing amounted to about 92 msec for low-probability RT and 62 msec for P300. These results suggest that P300 latency indexes some aspect of N_2O narcosis. Since current evidence favours the view that P300 latency measures stimulus evaluation processes (McCarthy and Donchin 1981; Magliero et al. 1984), the implication is that these processes are slowed by N_2O. This conclusion is consistent with the slowed processing model of inert gas narcosis (Fowler et al. 1985, 1986). This model proposes that the effects on performance of a gas such as N_2O can in large measure be explained by a slowing rather than a distortion of information processing. This slowing is thought to occur prior to stage processing and is attributed to a decrease

in the state of arousal. Consequently, stimulus evaluation processes should reflect this slowing, as was found in the present experiment.

Nitrous oxide also decreased P300 amplitude at both Cz and Pz but this decrease could not be strongly linked to either RT or P300 latency. The correlations between these variables and P300 amplitude were weak and P300 amplitude was relatively more variable. Moreover, unlike RT and P300 latency, the dose-response function for P300 amplitude reached an asymptote at 25%. This is an unusual pattern for N_2O but it probably cannot be dismissed as a peculiarity of this experiment because a similar pattern appears to be evident in a previous study (Fenwick et al. 1979). While these authors claimed that 10–40% N_2O decreased P300 amplitude linearly, their data could be interpreted as curvilinear, with an asymptote in the 20–40% range (Fenwick et al. 1979, Fig. 2). Another similarity between the results of the present study and those of Fenwick et al. is that the withdrawal of N_2O and the resumption of air breathing tended to increase P300 amplitude. This effect has also been noticed with broad-spectrum EEG activity (Williams et al. 1984).

Attempting to explain the decrease in P300 amplitude by invoking cognitive variables, such as a decrease in stimulus meaning or information transmission (Johnson 1986), is questionable at the moment. This is because the dose-response function for P300 amplitude does not show the usual pattern associated with the behavioural effects of N_2O (Fowler et al. 1985). It remains to be ascertained whether an early asymptote is the result of N_2O acting alone or is the result of a second factor acting in opposition to it. For example, the relatively small effect of N_2O on amplitude could be limited by a larger effect acting in the opposite direction linked to the high uncertainty associated with the low-probability stimulus (Johnson 1986). A further consideration is that ERP components earlier than P300 exhibit the normal dose-response function for N_2O (Jarvis and Lader 1971; Fenwick et al. 1979) while a later component, the contingent negative variation, is unaffected (Fenwick et al. 1979). This evidence raises the possibility that the effect of N_2O on the amplitude of an ERP component needs to be considered in relation to its temporal position in the wave form.

It is unlikely that the results of this experiment can be explained in terms of an ERP component other than P300. One possibility is that P200 movement potentials were synchronized with the slowing of RT. However, the component that was taken for P300 preceded RT by an average of 70 msec and was substantially smaller in the high-probability condition. Another possibility is that P300 and the slow wave (SW) were confounded and it was the latter that was influenced by N_2O. An inspection of Fig. 1 does not support this suggestion. The SW is evident in some subjects but not to the extent that it is seriously confounding the measurement of P300. It is also unlikely that the results of this experiment can be explained by the higher concentrations of oxygen in the N_2O conditions than in the air controls. Breathing 100% oxygen at atmospheric pressure does not influence RT and there is little evidence that it influences ERPs although P300 does not appear to have been investigated (Smith and Strawbridge 1974).

The results of this experiment have implications beyond N_2O narcosis. There is evidence suggesting that the behavioural effects of inert gases such as N_2O and hyperbaric nitrogen are identical, although their relative narcotic potencies differ (Fowler et al. 1985). Nitrogen narcosis is a danger to divers breathing compressed air and a factor which limits the depth to which they can dive safely. Past attempts to relate early ERP components to behavioural measures of nitrogen narcosis have been unsuccessful (Fowler and Ackles 1977). Therefore, it is of considerable theoretical and practical interest to determine whether P300 latency indexes nitrogen narcosis in the same manner as N_2O narcosis.

This study was funded by DCIEM Research Contract 97711-3-7449 to the first author and is DCIEM Research Report No. 87-P-49.

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